The *lpr* and *gld* genes in systemic autoimmunity: life and death in the *Fas* lane

Philip L. Cohen and Robert A. Eisenberg

The single gene lpr *and* gld *models of spontaneous systemic autoimmunity have attracted much attention in recent years. Here, Philip Cohen and Robert Eisenberg describe the fascinating recent findings that the* lpr *and* Fas *phenotypes result from defects in the* Fas *gene and, perhaps, in the ligand for fas, respectively.*

Mice homozygous for *lpr* (lymphoproliferation) develop a remarkable degree of lymphoid enlargement and progressive systemic lupus-erythematosus-like autoantibody formation[1]. In the prototype MRL/Mp-lpr/lpr strain, mice also develop vasculitis and immune complex glomerulonephritis, and half are dead by about five months. Lymphoproliferation, autoantibody production, and disease expression vary considerably in mice with other background genes. *Lpr^cg*, an independent mutation which is allelic to *lpr*, results in a phenotype indistinguishable from that caused by *lpr*. Mice homozygous for the generalized lymphoproliferative disease (*gld*), develop an identical illness, yet *lpr* and *gld* are non-allelic. Most of the immunological studies discussed below have only been done in *lpr*, but when they have been extended to *gld*, parallel results have been found, except with regard to the interaction of autoimmune (*lpr*, *gld*) and normal (±) cells in chimeras.

The *lpr* phenotype

The lymphoid enlargement in both strains is due to the massive accumulation of T cells. Despite lacking both CD4 and CD8, these cells bear certain markers generally found on activated T cells or on B cells, but do not express IL-2 receptors. They do express low levels of a polyclonal T-cell receptor (TCR) repertoire. Despite their activated appearance and phenotype, they are refractory to activation by antibodies to the TCR or by mitogens, and they are generally devoid of demonstrable function. Their relationship to autoantibody formation is indirect, and the two phenomena can be separated under certain experimental conditions. In addition to the dramatic T-cell abnormalities evident in *lpr* mice, recent studies have shown that the B cells are also abnormal and that, in the presence of *lpr* T cells in an *lpr* environment, only *lpr* B cells can produce autoantibodies[2,3]. Abnormalities of other cells of hematopoietic origin have also been described in *lpr* animals. Irradiation, followed by reconstitution with wild-type marrow, results in a normal phenotype.

lpr *and* fas

Until recently, the function of *lpr* has been obscure. The identification of *lpr* as the *Fas* gene, the product of which mediates a pathway for apoptosis, offers new insight into the mechanism of *lpr* autoimmunity, and underscores the relationship between apoptosis and autoimmunity also observed in the *Bcl-2* system[4].

Yonehara and colleagues observed that the binding of a monoclonal antibody to a human cell surface determinant, which they termed Fas, causes programmed cell death[5]. This ~35 kDa protein has a cytoplasmic anchor and is expressed in both lymphoid and non-lymphoid tissues. It has structural homology to the receptor for tumor necrosis factor (TNF), to the low-affinity receptor for nerve growth factor, and to the B-cell surface marker CD40 (Ref. 6). It is identical to APO-1, which is recognized by a monoclonal antibody made by another group[7]. The murine *Fas* gene was cloned and localized to chromosome 19 (Ref. 8). The demonstration by classical genetics that *lpr* mapped to the same area of this chromosome led to the successful test of the hypothesis that *lpr* represented a defect in the *Fas*-encoding gene[9,15]. *Lpr* mice probably have a genomic deletion of part of the *Fas* gene, and fail to produce a functional product. The molecular basis of the *lpr* Fas defect appears to be that multiple improperly spliced mRNAs are produced (M.F. Seldin, pers. commun.). As most of these fail to code for protein, there is, presumably, an absence of surface Fas. In contrast, mice with the allelic *lpr^cg* defect have a single base substitution, resulting in an amino acid substitution in the intracytoplasmic region. This alteration results in a failure to transmit the transmembrane signal for apoptosis.

The finding that *lpr* mice are deficient in a form of apoptosis provides a framework for understanding their defect, and a basis for further experimentation. The lack of a functional Fas cannot preclude other forms of apoptosis, as *lpr* mice are not notable for generalized developmental defects. Rather, the *lpr* defects are mainly manifest in the function of the immune system, over a period of months. One possible site of action of Fas is in thymic negative selection. For *lpr*, however, the expected deletion of superantigen-reactive T cells proceeds normally, even amongst the abnormal $CD4^-CD8^-$ cells[10,11]. Furthermore, *lpr* lymphocyte blasts are normally susceptible to apoptosis mediated by corticosteroids, by superantigens, or by killer T cells (P.L. Cohen, D. Leslie,

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R. Rapoport and R.A. Eisenberg, unpublished).

Further insights into the mechanism of *Fas* action come from analysis of the T-cell repertoire in *lpr* mice. Thymectomy at one month of age not only fails to retard the development of lymphadenopathy and autoimmune disease; it also fails to alter the V_β repertoire of single positive and double negative T cells four months later. This implies that the thymus is not an important site of Fas action (L. Herron, V. Kakkanaiah, P.L. Cohen, R.A. Eisenberg and B.L. Kotzin, unpublished).

The double-negative T cells in *lpr* mice appear to have descended from single-positive T cells, and their V_β repertoire implies that $CD8^+$ cells are the predominant ancestors (L. Herron *et al.*). It is likely that the failure to express a normal form of Fas leads to a defect in peripheral tolerance. Although much remains to be learned about Fas, it is possible that this surface receptor serves to eliminate self-reactive lymphoid cells as well as excess cells activated in the course of a normal immune response. The rise in *Fas* expression in individual T cells coincident with their activation is consistent with this latter notion[12]. In *lpr* mice, the absence of a functional Fas may thus lead to the gradual accumulation of lymphocytes that are normally deleted as a consequence of self tolerance and immunoregulation. For T cells, the downregulation of the TCR and of surface CD4 and CD8 may represent a secondary mechanism whereby these cells are inactivated, similar to what has been seen in transgenic mice with autoreactive TCRs[13]. A delay or inefficiency in this secondary mechanism might allow for sufficient aberrant autoantigen-reactive T cells to promote autoimmune disease. The preponderance of double-negative T cells of $CD8^+$ origin may reflect the greater need to downregulate potentially autoreactive $CD8^+$ than $CD4^+$ cells, as the former are potentially more dangerous, or it may relate to the greater importance of peripheral tolerance towards MHC class I-restricted molecules not expressed in the thymus. At the B-cell level, too, it is likely that *Fas* is important for peripheral tolerance. In *lpr* mice, the deficiency in Fas may lead to the emergence of self-reactive B-cell populations which are deleted in normal individuals.

The Fas ligand

The nature of the normal ligand for Fas is of critical importance in understanding the *lpr* mutation. The similarity of the *gld*-induced disease has led to speculation that the *gld* defect may represent a lack of functional ligand for the *lpr*-encoded receptor molecule. Fas-mediated signalling would, therefore, not occur, much as in *lpr* homozygotes. If this were the case, it would be expected that, in chimeric animals with both *gld* and normally lymphoid cells, the normal cells could supply the Fas ligand missing from *gld* cells and correct their disorder. Preliminary data suggest that this is indeed the case. Lethally irradiated *gld* mice reconstituted with equal numbers of normal and *gld* bone marrow cells show no evidence of excess polyclonal activation of *gld*-derived donor B cells[14]. These results contrast strongly with those from parallel experiments with *lpr* mice[2], and indicate an extrinsic defect in *gld* B cells, which may reflect the lack of a functional Fas ligand.

It appears that Fas, and potentially other molecules mediating apoptosis, have an intriguing role in the establishment and perpetuation of tolerance. It is clear that additional studies of the relationship between apoptosis and tolerance will yield insights into both basic immunology and autoimmunity.

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References

1 Cohen, P.L. and Eisenberg, R.A. (1991) *Annu. Rev. Immunol.* 9, 243–269
2 Sobel, E.S., Katagiri, T., Katagiri, K. *et al.* (1991) *J. Exp. Med.* 173, 1441–1449
3 Perkins, D.L., Glaser, R.M., Mahon, C.A. *et al.* (1990) *J. Immunol.* 145, 549–555
4 Strasser, A., Whittingham, S., Vaux, D.L. *et al.* (1991) *Proc. Natl Acad. Sci. USA* 88, 8661–8665
5 Yonehara, S., Ishii, A. and Yonehara, M. (1989) *J. Exp. Med.* 169, 1747–1756
6 Itoh, N., Yonehara, S., Ishii, A. *et al.* (1991) *Cell* 66, 233–243
7 Oehm, A., Behrmann, I., Falk, W. *et al.* (1992) *J. Biol. Chem.* 267, 10709–10715
8 Watanabe-Fukunaga, R., Brannan, C.I., Itoh, N. *et al.* (1992) *J. Immunol.* 148, 1274–1279
9 Watanabe-Fukunaga, R., Brannan, C.I., Copeland, N.G., Jenkins, N.A. and Nagata, S. (1992) *Nature* 356, 314–317
10 Kotzin, B.L., Babcock, S.K. and Herron, L.R. (1988) *J. Exp. Med.* 168, 2221–2229
11 Singer, P.A., Balderas, R.S., McEvilly, R.J. *et al.* (1989) *J. Exp. Med.* 170, 1869–1877
12 Owen-Schaub, L.B., Yonehara, S., Crump, W.L., III and Grimm, E.A. (1992) *Cell. Immunol.* 140, 197–205
13 Schönrich, G., Kalinke, U., Momburg, F. *et al.* (1991) *Cell* 65, 293–304
14 Sobel, E.S., Cohen, P.L. and Eisenberg, R.A. *Arthritis Rheum.* (Abstr.) (in press)
15 Allen, R.D., Marshall, J.D., Roths, J.B. and Sidman, C.I. (1990) *J. Exp. Med.* 172, 1367–1375